Research Note

Indole and Skatole in Fresh Pork as Possible Markers of Fecal Contamination†

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ABSTRACT

Zero-tolerance levels for fecal contamination on beef, pork, and poultry have been proposed because of outbreaks of foodborne illnesses associated with pathogenic microorganisms from this source. As a result, changes in the current meat inspection system will have to include means other than visual inspection of the carcass to ensure the absence of contamination. While the principal need is for rapid, in-plant microbial testing methods, there is also a need for chemical-instrumental methods. A rapid solid-phase extraction method was developed to measure indole and skatole in porcine meat using a gas chromatograph interfaced with a chemiluminescence detector. The minimum detectable level for both compounds was 10 ppb. Results from the analysis of contaminated pork showed that this approach may have limited value in the assessment of fecal contamination in pork samples, since not all of the samples had detectable levels of either indole or skatole.

Key words: Indole, skatole, pork, feces

In 1993, the U.S. Department of Agriculture Food Safety Inspection Service called for a zero-tolerance level for fecal contamination on beef carcasses and boneless beef *(1)*. Subsequently, there was significant pressure to expand this zero tolerance to pork and poultry *(2, 3)*. Current examination of meat for fecal contamination is based on organoleptic factors, sight and smell, which have been shown to be inadequate for the task of ensuring the absence of feces. Besides being an aesthetic issue, fecal contamination of meat is now considered both a regulatory and a safety problem because it is a form of adulteration that provides a means of transferring pathogenic bacteria from animal to man. The illnesses and deaths attributed to hemorrhagic *Escherichia coli* O517:H7 in undercooked hamburgers in the Washington State area in 1993 *(4)* and the subsequent publicity has brought this issue to the forefront of food safety concerns. Because of this and other studies that indicate the widespread occurrence of pathogenic microorganisms in meat, changes in the meat inspection system will most likely include means other than visual inspection of the carcass. These include verifying the food plant's use of hazard analysis critical control point systems and possible postslaughter microbial testing of the meat product. While the principal need is for rapid methods, especially for microbial testing that can be employed in slaughterhouses, there is also a need for chemical methods that have the potential to verify the results obtained from in-plant procedures. Without fecal contamination, the presence of coliforms like *E. coli* is not likely. Therefore, to aid in the detection and the potential control of feces-derived bacteria, we conducted research on the potential for developing a chemical method of analysis.

While there are many approaches available for investigation, our initial studies were carried out with the simple compounds indole and skatole (3-methylindole). These two compounds are metabolites of tryptophan normally found in the feces of animals such as cattle and pigs *(6)*. Either one or both compounds could potentially serve as a chemical marker for fecal contamination of meat. Skatole is the principal contributor to the characteristic odor of feces and has also been associated with "boar taint" odor in uncastrated male pigs *(5)*. Because of this association, several methods are available to determine indole and skatole in plasma, rumen fluid, back-fat, and feces *(9, 10, 11)*, but there are few available for their determination in meat tissue. Since indole and skatole have been found primarily in the adipose tissue, this is where most of the emphasis has been placed. Both Gibis et al. *(8)* and Garcia-Regueiro et al. *(7)* determined indole and/or skatole in porcine meat by high-performance liquid chromatography. Peleran and Bories *(12)* used a solvent extraction method in combination with gas chromatography (GC) and a nitrogen-specific thermionic detector for boar back fat. In this paper, we report a simple and rapid solid-phase extraction method for the isolation and

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quantitation of indole and skatole in fresh pork, using a GC interfaced with a more sensitive and specific nitrogen chemiluminescence detector, a Thermal Energy Analyzer™ (TEA) operating in the nitrogen mode.

MATERIALS AND METHODS

Materials

Indole and skatole were purchased from Sigma Chemical Co. (St. Louis, MO) and used without further purification. A GC working standard for both was prepared in dichloromethane (DCM) at a concentration of 1.0 μg/ml each. It was stored in a brown bottle at 4°C until used. Celite 545 (not acid washed), anhydrous sodium sulfate, pentane, hexane, and DCM were purchased from local suppliers as described previously *(13)*. Basic alumina and Florisil solid-phase extraction cartridges (SuperSpe-ed, 5 g) were purchased from Applied Separations (Allentown, PA). Fresh porcine meat samples that were visibly contaminated with feces and samples that were not contaminated were obtained from female carcasses from a local meat slaughter facility. These samples were trimmed of fat and then comminuted twice at room temperature through a 16-mm die plate prior to use. Fresh porcine feces were obtained from this same slaughter facility, and the bovine feces from a cattle raising facility within ARS.

Apparatus

The glass chromatographic columns, Kuderna-Danish (K-D) flasks, concentrator tubes and other equipment needed for this procedure have been described in detail elsewhere *(13)*.

Indole and skatole were determined with a Shimadzu gas chromatograph model GC-Mini3 connected to an external pyrolyzer interface controlled by a TEA model 610R Nitrogen Converter, which in turn is interfaced to a TEA model 502 chemiluminescence detector. The operating conditions were as follows: 1.8 m by 2.6 mm glass column packed with 5% SP-2401 DB on 100/120 mesh Supelcoport; He carrier gas, 35 ml/min; column operating isothermally, 140°C; injector, 220°C; pyrolyzer, 975°C; interface, 290°C; TEA vacuum, 1.0 mm; and a ThermoElectron Corp. CTR™ gas stream filter in place of a liquid nitrogen cold trap.

Determination

Feces. Indole and skatole were isolated from bovine and porcine feces by extracting 20.0 g of feces with 2 100-ml portions of DCM in a Virtis Virtishear™ followed by centrifugation at 3,000 rpm (Sorvall H1000B rotor) for 20 min at 0 to 5°C. The DCM was decanted through anhydrous sodium sulfate, concentrated to approximately 4 ml on a steam bath and then to 1 to 1.5 ml in a 70°C water bath. The sample was cleaned up on a 5-g Florisil solid-phase cartridge by transferring the sample concentrate to the cartridge with pentane:ether (80:20), adding an additional 50 ml of solvent, and collecting the eluant in a K-D setup. The eluate was concentrated to 1.0 ml and indole and skatole determined by GC-TEA. The presence of these two compounds in feces was confirmed by GC-MS by comparing their spectra to those from authentic standards.

Meat. Comminuted meat (10.0 g) was weighed into a glass mortar. Anhydrous sodium sulfate (25 g) was added, and the entire mixture ground with a pestle for 30 s. Celite (20 g) was then added to the mortar and the sample mixture gently ground for 15 to 20 s until the Celite was thoroughly mixed with the sodium sulfate and meat. The entire mixture was then ground an additional 1 to 2 min until the sample was a free-flowing mixture. The mixture was quantitatively transferred to the chromatographic column containing a glass wool plug on the bottom, and tamped with a glass rod to a total height of about 75 to 80 mm. Anhydrous sodium sulfate (30 g) was then added to the top of the column. The mortar and pestle were rinsed with 15 ml of DCM, which was added to the column. An additional 160 ml of DCM was immediately added to the column. The eluate was collected in a 250-ml K-D flask equipped with a 10-ml concentrator tube. When the column stopped dripping, the K-D flask was removed and 2 small boiling chips were added. A 3-section Snyder column was placed on top of the K-D flask, and the sample concentrated on a steam bath to approximately 4 ml. One ml of hexane was added to the concentrator tube and the sample concentrated to 1 to 3 ml (or until only hexane and fat remain) with a micro-Snyder column in a 70°C water bath. Using a disposable glass pipet, the sample was transferred to a basic alumina cartridge that had been prewashed with 30 ml of pentane (precaution: do not let column run dry). The concentrator tube was rinsed with 2 4-ml portions of pentane and the rinse transferred to the column. The column was washed with 3 30-ml portions of pentane and the eluant collected in a 250-ml flask; the pentane was discarded. The analytes were eluted with 3 30-ml portions of DCM into a K-D flask with a 4-ml concentrator tube attached. After elution, the sample was concentrated on a steam and water bath to 1.0 ml. The indole and skatole were determined on a GC-TEA using a 9.0 μl injection. Minimum detectable level (signal:noise, >2) was 10 ppb for indole and skatole.

Since indole and skatole were reportedly light sensitive, their stability in DCM was tested by taking an aliquot of the GC working standard and storing it in a clear glass concentrator tube subjected to room light and ambient temperature for 72 h. We found that their concentration did not decrease by more than 1%. A DCM extract from a spiked meat sample was also stored for 72 h. at room temperature; similar results were obtained. The GC working standard showed no signs of deterioration after long-term storage (6 months) when compared to a freshly prepared standard.

RESULTS AND DISCUSSION

To determine if indole or skatole could serve as potential chemical markers for fecal contamination, bovine and porcine feces were analyzed for their presence. Bovine feces ($n = 6$) contained indole ranging from 0.02 to 0.83 ppm (mean, 0.31 ppm), but only trace amounts of skatole (<0.01 ppb). Porcine feces ($n = 5$) contained both compounds; indole ranging from 0.70 to 9.41 ppm (mean, 2.51 ppm) and skatole ranging from 4.66 to 13.80 ppm (mean, 10.43 ppm). Dehnhard et al. *(6)* also found that skatole concentrations were lower in feces from ruminants than those from monogastrics.

The use of indole or skatole as a chemical marker for fecal contamination requires that they be unique to feces. More importantly, their use as a chemical marker also requires that they be present in sufficient concentrations in contaminated meat that they be readily detected. Since the mean concentrations of these two indole compounds were low in bovine feces, their use as a chemical marker in beef would result in nondetectable levels even in contaminated beef because of the significant dilution factor. We decided, however, to assess their potential as chemical markers for a laboratory-based procedure to confirm the presence of fecal contamination of fresh pork meat. The probability of indole and skatole being present was considered high since both compounds have been found in the intestinal tract of pigs *(15)*.

TABLE 1. *Indole and skatole in fresh porcine meat contaminated or not with feces*

Sample no.	Contaminated	Indole[a], (ppb)	Skatole[a], (ppb)
1	+	268	ND[b]
2	+	186	113
3	+	ND	102
4	+	ND	108
5	+	ND	14
6	−	ND	ND
7	−	ND	ND
8	−	ND	ND

[a] Average of duplicate determinations.
[b] ND, none detected: <10 ppb.

Initially, several samples of fresh pork known to be free from fecal contamination were checked for the presence of indole and skatole by our method; neither was detected. A spiked recovery study in which both compounds were added to meat ($n = 6$) at the 100 ppb level was then carried out. Indole was recovered at the 82.4% ± 5.9% level, and skatole at 85.5% ± 4.5% level. Next, pork samples that were either visibly contaminated or were free of feces were analyzed. The results are shown in Table 1. Neither compound was detected in the 3 samples that were visibly free from fecal contamination. In the 5 samples that had visible contamination, either indole or skatole was detected. Skatole was found in 4 of 5 samples, while indole was found in 2 of 5. Only one sample had both indole and skatole. The greater prevalence of skatole in the meats analyzed is consistent with literature reports which show that skatole is more abundant in fresh feces than indole *(14, 16)*. As a result, contaminated meat would be expected to contain higher levels of skatole. Although the method is sensitive to 10 ppb for both indole and skatole, one contaminated sample did not contain any trace of skatole, but did have a significant amount of indole. This indicated that it is necessary to screen for both compounds, and not just for skatole, if fecal contamination of pork is to be confirmed by this method.

CONCLUSION

The results suggest that this approach, analyzing for indole and skatole, may have limited value as a definitive chemical marker test for fecal contamination. However, the solid-phase extraction method described in this paper is a rapid means to determine indole and skatole in fresh porcine meat, and it is likely to be applicable to other types of samples.

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